SHIMADZU

ThP 591

Simultaneous quantitation of N-nitrosamines and NDSRI in API and formulation by using a DUIS ionization source in LC-MS/MS

Nitish Ramchandra Suryawanshi[1]; Samruddha Chavan[1]; Nitin Shukla[1] ; Devika Tupe[1]; Siddhesh Ghadi[1]; Ramesh Manigiri[1]; Shalu Nair[1]; Jitendra Kelkar[1]; Pratap Rasam[1]

[1]Shimadzu Analytical (India) Pvt. Ltd., 1 A/B Rushabh Chambers, Makwana Road, Marol, Andheri (E), Mumbai-400059, Maharashtra, India.

1. Overview

N-nitrosamine impurities, are organic compounds of the chemical structure $R_2N{-}N{=}O$, where R is usually an alkyl group. These compounds are listed as Class 1 mutagens in ICH M7[1]. N-nitrosamines have been monitored extensively in pharmaceuticals since 2018. Since 2020, there has been an increased number of reports of more structurally complex N-nitrosamines related to the structure of the active substance itself in several drug products. These are often referred to as “nitrosamine drug substance related impurities” (NDRSIs) and are generally formed by nitrosation of an amine moiety present in the active substance. To date, secondary amine functional groups seem most vulnerable to form corresponding N-nitrosamines[2]. After N-nitrosamines, the US Food and Drug Administration (FDA) now demands the pharmaceutical manufacturers to establish if their active pharmaceutical substance (API) or formulation is free from NDSRIs. Considering the risk associated & regulatory implications, there is an increasing demand for testing of both these type of molecules in API and formulations.

2. Introduction

N-nitrosamines are typically monitored using atmospheric pressure chemical ionization (APCI) in LCMS analysis. This preference is due to their smaller molecular size, which can result in higher background interference when using electrospray ionization (ESI). In contrast, NDSRIs are primarily analyzed using ESI because they exhibit higher sensitivity with this ionization method. Considering the above scenario, API and formulations with potential of having presence of both N-nitrosamines and NDSRIs need to be tested with multiple methods. Consumption of chemicals, reagents and decreasing throughput are some of the drawbacks associating with such multiple testing. This leads to a high demand for having a method with simultaneous detection of both N-nitrosamines and NDSRI using LC-MS/MS. For simultaneous detection, a dual ionization source (DUIS) can be very useful as it can ionize both the N-nitrosamines and NDSRIs using same interface. Optimum sensitivities for individual compounds can be achieved by optimizing the source conditions to either have ESI like or APCI like conditions. This poster describes a partially-validated LC-MS/MS procedure for quantitation of nine N-nitrosamines and NDSRI in Afatinib API and formulation which was performed using an Ultra High Performance Liquid Chromatograph (UHPLC) Nexera™ X3 coupled with an LCMS-8060NX, a Triple Quadrupole Mass Spectrometer equipped with a DUIS source from Shimadzu Corporation, Japan (Figure 1). The nine N-nitrosamines and one NDSRI includes N-nitroso-dimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-Nitrosopyrrolidine (NPYR), N-nitroso-diethylamine (NDEA), N-nitrosopiperidine (NPIP), N-nitroso-ethyl-isopropylamine (NEIPA), N-nitroso-diisopropylamine (NDIPA), N-nitroso-dipropylamine (NDPA), N-nitroso-dibutylamine (NDBA) and N-nitroso Afatinib impurity-2 (N-AFA).

3. Methods

3-1. LC-MS/MS analysis

Individual standards for all nine N-nitrosamines and NDSRI were purchased locally. Stock solutions for individual N-nitrosamines and NDSRI were prepared and analysed in scan mode. Further, steps such as precursor ion selection, Multiple Reaction Monitoring (MRM) optimization at different Collision Energies (CE) and voltage optimization were performed using Shimadzu’s LabSolutions auto MRM optimization feature to obtain MRMs and their optimum CEs. To achieve optimum sensitivity, parameters such as interface voltage & focus voltage were fine tunned. An LC method (Table 1) was developed with an aim to separate 10 compounds and API under study (Figure 2) which was achieved using Shimadzu make Shim-pack Scepter Claris PFPP (Metal free), 150 mm x 2.1 mm I.D. and 3.0 µm LC column (P/N: 227-31214-05). Optimized MRMs for individual compounds are listed in table 2. For quantitation, a linearity ranging from 1.0-20.0 ppb for NDMA, 0.5-20.0 ppb for NMEA, NPYR, NPIP, NDEA, NEIPA, NDIPA, NDPA, NDBA and 0.5-7.5 ppb for N-AFA was prepared in diluent and was analyzed using LC-MS/MS. The limit of quantitation (LOQ) was found to be 1.0 ppb for NDMA and 0.5 ppb for rest of the compounds. The S/N and % RSD at LOQ are shown in table 3.

Figure 1: Shimadzu Nexera™ X3 UHPLC coupled with an LCMS-8060NX Triple quadrupole mass spectrometer

3-2. Analytical conditions

Table 1. Instrument parameters for LC-MS/MS

HPLC System	: Nexera™ X3
Column	: Shim-pack Scepter PFPP-120, 3.0 µm 2.1 x 150 mm (P/N :227-31214-05)
Column Oven Temp.	: 45 ° C
Mobile Phases	: A:10 mM Ammonium formate in LC-MS grade water B:LC-MS grade methanol: LC-MS grade Acetonitrile (9:1) v/v
Flow Rate	: 0.45 mL/min
Gradient Program (B%)	: 0-3 min → 2 (%); 3-8 min → 40 (%); 8-20 min → 70 (%); 20-21 min → 100 (%); 21-25 min → 100 (%) 25-25.5 min → 2 (%) 35 min → STOP.
Injection Volume	: 40 µL
LC-MS System	: LCMS™-8060NX
Ionization Source	: DUIS
LC-MS Temperature	: Interface: 400° C Desolvation Line: 250° C Heater Block: 400° C
LC-MS Gas Flows	: Nebulizing Gas: 3.0 L/min Drying Gas: 4.0 L/min

Table 2: MRM transitions for 9 N-nitrosamines and 1 NDSRI

Compound	Precursor m/z	Product m/z	CE
NDMA	74.90	43.05	-10
NMEA	89.20	61.25	-15
NPYR	101.00	55.25	-18
NDEA	103.00	29.05	-16
NPIP	115.05	41.00	-22
NEIPA	117.00	75.10	-13
NDIPA	131.10	89.15	-12
NDPA	131.00	89.05	-12
NDBA	159.00	41.05	-22
N-AFA	501.05	414.00	-23

3-3. Sample preparation

- **Sample:** For API sample, weigh 10 mg of API and for formulation sample, weigh 10 mg equivalent weight of formulation in a 15 mL of centrifuge tube. Add 5 mL of diluent to it, sonicate for 2 mins. Filter the solution using 0.22 µm nylon filter and analyze the filtrate using LC-MS/MS.
- **Spiked sample:** For API sample, weigh 10 mg of API and for formulation sample, weigh 10 mg equivalent weight of formulation in a 15 mL of centrifuge tube. Spiked the standard mix to it to make the spiking concentration of 1.0 ppb for NDMA and 0.5 ppb for rest of the compounds. Add 5 mL of diluent to it, sonicate for 2 mins. Filter the solution using 0.22 µm nylon filter and analyze the filtrate using LC-MS/MS.

4. Results and Discussion

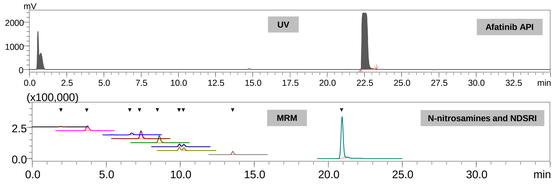

Figure 2: UV and MS chromatogram of Afatinib API spiked sample.

Figure 3 & 4 depicts the calibration curve and LOQ level standard (Representative chromatograms of 3 compounds)

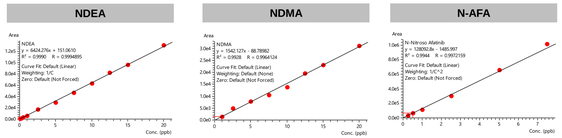

Figure 3: Calibration curves for NDEA, NDMA & N-AFA as representative compounds

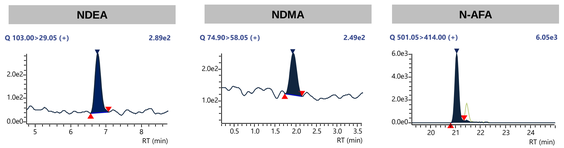

Figure 4: Chromatograms for 0.5 ppb NDEA, 1.0 ppb NDMA & 0.5 ppb N-AFA as representative compounds

Table 3: Coefficient of determination for calibration curves, repeatability of area for LOQ solution and S/N ratio for LOQ solution (Conc. expressed are as such)

Sr. No.	Abbr.	r^2	CC Range (ppb)	LOQ		
				Conc. (ppb)	% RSD (n=6)	S/N
1	NDMA	0.993	1.0-20.0	1.0	11.0	30
2	NMEA	0.999	0.5-20.0	0.5	8.2	52
3	NPYR	0.998	0.5-20.0	0.5	12.3	66
4	NDEA	0.999	0.5-20.0	0.5	11.0	89
5	NPIP	0.999	0.5-20.0	0.5	7.4	77
6	NEIPA	0.999	0.5-20.0	0.5	4.9	198
7	NDIPA	0.999	0.5-20.0	0.5	11.3	101
8	NDPA	0.999	0.5-20.0	0.5	8.5	86
9	NDBA	0.999	0.5-20.0	0.5	4.0	142
10	N-AFA	0.994	0.5-20.0	0.5	8.4	609

Abbr. = Abbreviation; CC = Calibration curve; Conc. = Concentration; S/N = Signal-to-noise ratio

Summary of amount obtained in API and formulation sample are shown in table 4 and summary for % recovery are shown in Table 5.

Table 4: Summary of concentrations obtained in Afatinib API, and formulation samples are shown in.

Content in Afatinib sample (ppb)		
Compounds	**API**	**Formulation**
NDMA	Below LOQ	Below LOQ
NMEA	Below LOQ	Below LOQ
NPYR	Below LOQ	Below LOQ
NDEA	Below LOQ	Below LOQ
NPIP	Below LOQ	Below LOQ
NEIPA	Below LOQ	Below LOQ
NDIPA	Below LOQ	Below LOQ
NDPA	Below LOQ	Below LOQ
NDBA	Below LOQ	Below LOQ
N-AFA	Below LOQ	Below LOQ

Table 5: Summary for samples spiked at 500 ppb for NDMA and 250 ppb for rest of the compounds (Results expressed are relative to sample concentration)

% Recoveries of N-nitrosamines and Nitroso Afatinib in Afatinib API				
Comp.	**Amt. in sample (ppb)**	**Amt. obtained (ppb)**	**Amt. spiked (ppb)**	**% Recovery**
NDMA	Below LOQ	433.7	500	87
NMEA	Below LOQ	252.8	250	101
NPYR	Below LOQ	304.4	250	122
NDEA	Below LOQ	292.1	250	117
NPIP	Below LOQ	252.8	250	101
NEIPA	Below LOQ	251.7	250	101
NDIPA	Below LOQ	250.0	250	100
NDPA	Below LOQ	294.1	250	118
NDBA	Below LOQ	253.3	250	101
N-AFA	Below LOQ	219.6	250	88

5. Conclusion

- Quantitation of 9 N-nitrosamines and NDSRI in Afatinib API and formulation was successfully demonstrated on Shimadzu LCMS-8060NX equipped with DUIS source.
- Repeatability for N-nitrosamines and NDSRI was found to be less than 15.0 %.
- Recoveries for all N-nitrosamines and NDSRI were found to be between 70-130 %.
- The newly developed IonFocus™ ion source unit and patented lens system (UF-Qarray II) of LCMS-8060NX improves system robustness by efficiently introducing only ions into the mass spectrometer and removing unwanted neutral particles and contaminants.
- Revolutionary DUIS ion source with ion focus technology not only detect compounds of diverse nature but also improves baseline by eliminating contaminants.

6. References

[1] WHO Information Note; Update on Nitrosamine impurities, 20 Nov. 2019.
[2] Răzvan C. Cioc et al, 2023, Formation of N-Nitrosamine Drug Substance Related Impurities in Medicines: A Regulatory Perspective on Risk Factors and Mitigation Strategies, ACS Publications.